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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	04

Application Number	10/593,384-Conf. #
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First Named Inventor	Ajit Lalvani
Art Unit	N/A
Examiner Name	Not Yet Assigned
Attorney Docket Number	54111/HO-P03388US0-10612116

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
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- [ ] Information Disclosure Statement
- [X] Certified Copy of Priority Document(s)
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- [ ] Drawing(s)
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Certificate of Mailing (1 page)
Statement (2 pages)
1 Return Postcard

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	FULBRIGHT & JAWORSKI L.L.P.		
Signature			
Printed name	Melissa W. Acosta		
Date	April 19, 2007	Reg. No.	45,872

Docket No.: 54111/HO-P03388US0-10612116
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Ajit Lalvani

Application No.: 10/593,384 | Confirmation No.: Not Yet Assigned

Filed: March 21, 2005 | Art Unit: N/A

For: DIAGNOSTIC TEST | Examiner: Not Yet Assigned

CLAIM FOR PRIORITY AND SUBMISSION OF PRIORITY DOCUMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants hereby claim priority under 35 U.S.C. 119 based on the following prior foreign applications filed in the following foreign country on the date indicated:

Country	Application No.	Date
GB	0406271.7	19 March 2004

In support of this claim, a certified copy of the said original foreign application is filed herewith.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 06-2375, under Order No. 54111/HO-P03388US0-10612116 from which the undersigned is authorized to draw.

Dated: April 19, 2007

Respectfully submitted,

By _____

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I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with patent application GB0406271.7 filed on 19 March 2004.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

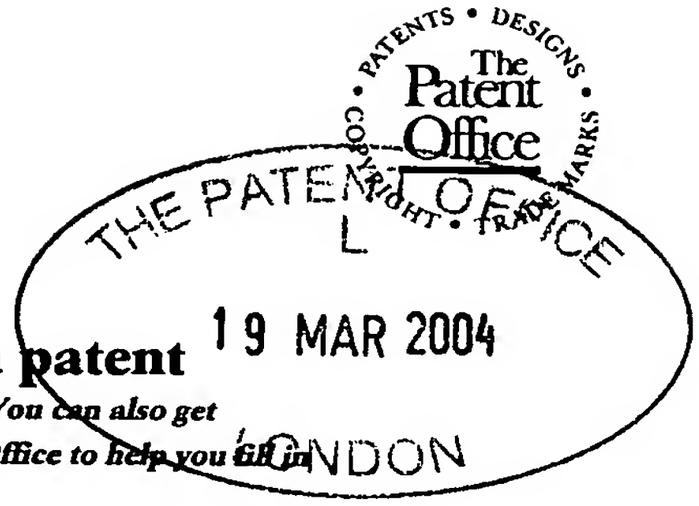
Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 2 April 2007

Patents Form 1/77

Patents Act 1977
(Rule 16)



22MAR04 E882557-1 D00192
P01/7700 0.00-0406271.7 NONE

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference — P91297 JCI

2. Patent application number *(The Patent Office will fill this part in)* — 0406271.7 — 19 MAR 2004

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Isis Innovation Limited
Ewert House, Ewert Place
Summertown, Oxford, OX2 7SG
United Kingdom

Patents ADP number *(if you know it)* 39985640007

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — DIAGNOSTIC TEST

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

J. A. KEMP & CO.

14 South Square
Gray's Inn
London
WC1R 5JJ

Patents ADP number *(if you know it)* 26001

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months.

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute (see note f)

Number of earlier UK application	Date of filing *(day / month / year)*

8. Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?

Answer YES if:

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.

Otherwise answer NO (See note d)

Yes

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form	-
Description	26
Claim(s)	4
Abstract	0
Drawing(s)	3 +3

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	-
Request for a preliminary examination and search (Patents Form 9/77)	-
Request for a substantive examination (Patents Form 10/77)	-
Any other documents (please specify)	-

11. I/We request the grant of a patent on the basis of this application.

Signature(s) J.A. KEMP & CO. Date 19 March 2004

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

IRVINE, Jonquil Claire
020 7405 3292

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered YES in part 8, a Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) Part 7 should only be completed when a divisional application is being made under section 15(4), or when an application is being made under section 8(3), 12(6) or 37(4) following an entitlement dispute. By completing part 7 you are requesting that this application takes the same filing date as an earlier UK application. If you want the new application to have the same priority date(s) as the earlier UK application, you should also complete part 6 with the priority details.

DIAGNOSTIC TEST

Field of the Invention

The invention relates to a method of diagnosis of *Mycobacterium tuberculosis* infection in a human. It also relates to peptide compositions and a kit which can be used to carry out the diagnostic method.

Background to the Invention

Accurate diagnosis of tuberculosis infection is essential for the treatment, prevention and control of this resurgent disease. Since *Mycobacterium tuberculosis* (MTB) is often difficult to culture from patients with active TB, and impossible to culture from healthy latently infected people, an immune-based diagnostic test indicating the presence or absence of MTB infection would be very useful for diagnosis of active TB and screening for latent MTB infection.

The only widely used test is the century-old tuberculin skin test (TST) or Mantoux test which is based on the detection of a delayed type hypersensitivity (DTH) response to an intradermal administration of a Purified Protein Derivative of the mycobacterium. This test has many drawbacks foremost amongst these is its poor specificity which results from the broad antigenic cross-reactivity of purified protein derivative (PPD), a crude mixture of over two hundred MTB proteins widely shared between MTB, *M. bovis Bacillus Calmette-Guerin* (BCG) and most environmental mycobacterial. Hence, false-positive results are common in people with environmental mycobacterial exposure and previous BCG vaccination. This presents a significant problem because most of the world's population is BCG-vaccinated and the confounding effect of BCG persists for up to 15 years after vaccination.

Comparative genomics has identified several genetic regions in MTB and *M. bovis* that are deleted in *M. bovis BCG*. Several regions of difference, designated RD1 - RD16, between MTB or *M. bovis* and BCG have been identified. All represent parts of the *M. bovis* genome deleted during prolonged *in vitro* culture. RD-1 was deleted before 1921, when BCG was first disseminated internationally for use as a vaccine. RD-1 is thus absent

from all vaccine strains of BCG, as well as most environmental mycobacteria, but is still present in the *Mycobacterium tuberculosis* complex, including all clinical isolates of MTB and *M. bovis*. There are nine open reading frames (ORFs) in the RD1 gene region. Early secretory antigen target-6 (ESAT-6) and culture filtrate protein 10 (CFP10) are encoded in RD-1 and have been intensively investigated in animal models and humans over the last few years. ESAT-6 and CPF10 are strong targets of the cellular immune response in animal models, tuberculosis patients and contacts and so may be used in new specific T cell-based blood tests which do not cross-react with BCG.

Cellular immune responses to gene products from RD1, RD2 and RD14 have recently been investigated in *M bovis*-infected and BCG-vaccinated cattle. Eight antigens were deemed to be potent T cell antigens, Rv1983, Rv1986, Rv3872, Rv3873, Rv3878, Rv3879c, Rv1979c, and Rv1769) (Cockle *et al.*, 2002, Infect. Immun. 70:6996-7003). However it is not possible to predict based on the antigens which are T-cell antigens in cattle which will be T-cell antigens in humans. As well as other differences in antigen processing, presentation and recognition, cattle have different MHC molecules from humans, and thus are expected to recognise different antigens.

Summary of the invention

The present inventors have identified Rv3879c as a major T-cell antigen in humans, with 45% if tuberculosis patients responding to peptides from the Rv3879 gene product. Only one of 38 (2.6%) BCG-vaccinated donors responded to peptides from Rv3879c. The highly specificity of Rv3879c peptides, together with their moderate sensitivity in tuberculosis patients, identify these peptides as candidates for inclusion in new T cell-based tests for MTB infection.

Crucially, the inventors identified 3 individuals (out of 49 culture confirmed TB patients) who responded to Rv3879c peptides and who did not respond to any of 35 overlapping 15mer peptides spanning the length of ESAT-6 and CFP10 (which are known to be immunodominant MTB antigens of diagnostic utility). This result shows that Rv3879c peptides can be used to increase the sensitivity of diagnostic tests which use ESAT-6 and CFP10 peptides. This increase in sensitivity (which was 6% in the present

study of 49 TB patients) is clinically very important. A very high sensitivity allows doctors to rule out the possibility of tuberculosis when a diagnostic test is negative. In particular immune based diagnostic tests (including the in vivo skin test) may give false negative results in immunosuppressed individuals because of their limited sensitivity. A higher diagnostic sensitivity will allow doctors to accurately detect TB infection even in these vulnerable immunosuppressed patients who are at the highest risk of severe and disseminated tuberculosis.

Accordingly, the invention provides a method of diagnosing *Mycobacterium tuberculosis* infection in a human, or of determining whether a human has been exposed to *Mycobacterium tuberculosis*, comprising:

(i) contacting T-cells from said human with one or more of

(a) a peptide having the sequence shown in SEQ ID NO: 1;

(b) a peptide having or comprising the sequence of at least 8 consecutive amino acids of the sequence shown in SEQ ID NO: 1; or

(c) a peptide having or comprising a sequence which is capable of binding to a T-cell receptor which recognises a peptide as defined in (a) or (b); and

(ii) determining whether any of the said T-cells recognise said peptide.

Brief Description of the Figures

Figure 1 shows the proportion of culture-confirmed TB patients (n=49) and healthy, unexposed BCG vaccines (n=38) responding in IFN-γ-ELISPOT to peptide pools from the four RD region gene products. PBMCs from each participant were tested using the IFN-γ-ELISPOT assay with peptide pools of between 5 and 7 peptides representing different antigens from RD1 (Rv 3873, Rv3878, Rv3879c) and RD2 (Rv1989c).

A: Percentage of culture confirmed TB patients and unexposed BCG vaccinees who responded to each peptide pool in IFN-γ-ELISPOT.

B: Percentage of culture confirmed TB patients and unexposed BCG vaccinees who responded to one or more peptide pools from each of the RD1 and RD2 gene products. The right hand-most column shows the percentage of donors who responded to one or

more of any of the 11 peptide pools from the 4 antigens. The solid columns show response rates in TB patients, and the hatched columns show response rates in unexposed BCG-vaccinated donors.

Figure 2 shows the magnitude of IFN-γ ELISPOT responses to RD region antigens in 49 culture confirmed TB patients (A) and 38 healthy, unexposed BCG vaccinees (B). Frequencies of peptide-specific IFN-γ-secreting spot-forming cells (SFCs) summated for each of the constituent peptide pools for each antigen, enumerated by *ex vivo* ELISPOT assay in patients with TB(A), and healthy, unexposed BCG vaccinated donors(B). The horizontal bars represent the median response for each antigen. Points on the baseline represent individuals with no response to a given antigen (ie less than 5 SFCs above the negative control for each of the constituent peptides of each pool of the given antigen). The broken horizontal line represents the predefined cutoff point (5 SFC per 2.5 x 10^5 PBMC, which translates into a threshold of detection of 20 peptide-specific T-cells per million PBMC).

Figure 3 illustrates the location and homology of PPE protein family motif as described (http://genolist.pasteur.fr/TubercuLIST/mast/P210.1.html), within the partial amino acid sequence of Rv3873 (amino acid residues 100-160). Amino acid residues are shown in the one letter code. Underlined residues indicate the given peptide sequence. Identical residues are indicated with a cross.

Detailed description of the invention

The invention concerns diagnosis of tuberculosis infection in a human based on determination of whether the T cells of the human recognise an epitope of Rv3879c (SEQ ID NO:1). The method may also comprise determining whether T-cells of the human recognise one or more further *Mycobacterium tuberculosis* T-cell antigen(s), such as antigens encoded by the RD-1 or RD-2 region (preferably ESAT-6 and/or CFP10). In one embodiment the method comprises determining whether the T cell recognise one or more of the peptides represented by SEQ ID NO's 2 to 18.

The human who is tested typically has an active or latent mycobacterial infection, or has had such an infection recently. The human may test positive or negative in a

Mantoux test. The human may be at risk of a mycobacterial infection, typically for socio-economic reasons or may have a genetic or acquired predisposition to mycobacterial infection.

The human may be a known or suspected contact who has been exposed to or may have been exposed to *Mycobacterium tuberculosis*. Typically the exposure is to pulmonary tuberculosis, such as 'open' pulmonary tuberculosis which is sputum A.F.B. (acid-fast bacillus) smear positive. Thus the method may be used to trace the healthy contacts of individuals with such tuberculosis infections. The method may also be used to carry out population surveys to measure the number of individuals in a population who have a *Mycobacterium tuberculosis* infection. The contact may be someone whose exposure is a household, work place (such as a health care worker) or prison exposure (such as a prisoner). The exposure may have resulted from residing in a country with high prevalence of TB, and diagnostic testing after emigration to a country with a low prevalence of TB. Thus the contact may be an immigrant.

The human who is tested (who has a known or suspected exposure) may be healthy or might have a chronic condition putting them at a higher risk of developing active TB and/or which may make TB infection harder to diagnose. Examples include HIV infected individuals, individuals taking immunosuppressants (e.g. corticosteroids, azathioprine and anti-TNF-α agents, such as infliximab, and cancer therapy), hemodialysis patients, organ transplant recipients, diabetics and very young children (aged under 5 years old, particularly under 2 years old).

The T cells which recognise the peptide in the method are generally T cells which have been pre-sensitised *in vivo* to antigen from a *M. tuberculosis*. These antigen-experienced T cells are generally present in the peripheral blood of a host which has been exposed to the *M. tuberculosis* at a frequency of 1 in 10^6 to 1 in 10^3 peripheral blood mononuclear cells (PBMCs). The T cells may be CD4 and/or CD8 T cells.

In the method the T cells can be contacted with the peptides *in vitro* or *in vivo*, and determining whether the T cells recognise the peptide can be done *in vitro* or *in vivo*. Thus the invention provides a method of diagnosis which is practised on the human body.

Determination of whether the T cells recognise the peptide is generally done by

detecting a change in the state of the T cells in the presence of the peptide or determining whether the T cells bind the peptide (e.g. using an MHC tetramer combined with FACS analysis system), i.e. the method of the invention does not necessarily rely on the detection of a functional response of the T cell.

In the case where a change in state of the T cells is detected this is generally caused by antigen specific functional activity of the T cells after the T cell receptor binds the peptide. Generally when binding the T cell receptor the peptide is bound to an MHC class I or II molecule, which is typically present on the surface of an antigen presenting cell (APC).

The change in state of the T cell may be the start of or increase in secretion of a substance from the T cell, such as a cytokine, especially IFN-γ, IL-2 or TNF-α. Determination of IFN-γ secretion is particularly preferred. Intracellular cytokine detection by FACS may be used. The substance can typically be detected by allowing it to bind to a specific binding agent and then measuring the presence of the specific binding agent/substance complex. Detection of the substance may be carried out using an ELISA based system. The specific binding agent is typically an antibody, such as polyclonal or monoclonal antibodies. Antibodies to cytokines are commercially available, or can be made using standard techniques.

Typically the specific binding agent is immobilised on a solid support. After the substance is allowed to bind the solid support can optionally be washed to remove material which is not specifically bound to the agent. The agent/substance complex may be detected by using a second binding agent which will bind the complex. Typically the second agent binds the substance at a site which is different from the site which binds the first agent. The second agent is preferably an antibody and is labelled directly or indirectly by a detectable label.

Thus the second agent may be detected by a third agent which is typically labelled directly or indirectly by a detectable label. For example the second agent may comprise a biotin moiety, allowing detection by a third agent which comprises a streptavidin moiety and typically alkaline phosphatase as a detectable label.

In one embodiment the detection system which is used is the *ex-vivo* ELISPOT

assay described in WO 98/23960. In that assay IFN-γ secreted from the T cell is bound by a first IFN-γ specific antibody which is immobilised on a solid support. The bound IFN-γ is then detected using a second IFN-γ specific antibody which is labelled with a detectable label. Such a labelled antibody can be obtained from MABTECH (Stockholm, Sweden). Other detectable labels which can be used are discussed below.

The change in state of the T cell which can be measured may be the increase in the uptake of substances by the T cell, such as the uptake of thymidine. The change in state may be an increase in the size of the T cells, or proliferation of the T cells, or a change in cell surface markers on the T cell.

Generally the T cells which are contacted in the method are taken from the host in a blood sample, although other types of samples which contain T cells can be used. The sample may be added directly to the assay or may be processed first. Typically the processing may comprise diluting of the sample, for example with water or buffer. Typically the sample is diluted from 1.5 to 100 fold, for example 2 to 50 or 5 to 10 fold.

The processing may comprise separation of components of the sample. Typically mononuclear cells (MCs)are separated from the samples. The MCs will comprise the T cells and APCs. Thus in the method the APCs present in the separated MCs can present the peptide to the T cells. In another embodiment only T cells, such as only CD4 or only CD8 T cells, can be purified from the sample. PBMCs, MCs and T cells can be separated from the sample using techniques known in the art, such as those described in Lalvani *et al* (1997) *J.Exp. Med.* **186**, p859-865.

Preferably the T cells used in the assay are in the form of unprocessed or diluted samples, or are freshly isolated T cells (such as in the form of freshly isolated MCs or PBMCs) which are used directly *ex vivo*, i.e. they are not cultured before being used in the method. However the T cells can be cultured before use, for example in the presence of one or more of the peptides, and generally also exogenous growth promoting cytokines. During culturing the peptides are typically present on the surface of APCs, such as the APC used in the method. Pre-culturing of the T cells may lead to an increase in the sensitivity of the method. Thus the T cells can be converted into cell lines, such as short term cell lines (for example as described in Ota *et al* (1990) *Nature* **346**, p183-187).

The APC which is typically present in the method may from the same host as the T cell or from a different host. The APC may be a naturally occurring APC or an artificial APC. The APC is a cell which is capable of presenting the peptide to a T cell. It is typically a B cell, dendritic cell or macrophage. It is typically separated from the same sample as the T cell and is typically co-purified with the T cell. Thus the APC may be present in MCs or PBMCs. The APC is typically a freshly isolated *ex vivo* cell or a cultured cell. It may be in the form of a cell line, such as a short term or immortalised cell line. The APC may express empty MHC class II molecules on its surface.

Typically in the method the T cells derived from the sample can be placed into an assay with all the peptides (i.e. a pool of the peptides) which it is intended to test (the relevant panel) or the T cells can be divided and placed into separate assays each of which contain one or more of the peptides. Preferably in the *in vitro* or *in vivo* forms of the method.

The invention also provides the peptides such as two or more of any of the peptides mentioned herein (for example in any of the combinations mentioned herein) for simultaneous, separate or sequential use (eg. for *in vivo* use).

In one embodiment peptide *per se* is added directly to an assay comprising T cells and APCs. As discussed above the T cells and APCs in such an assay could be in the form of MCs. When peptides which can be recognised by the T cell without the need for presentation by APCs are used then APCs are not required. Analogues which mimic the original peptide bound to a MHC molecule are an example of such a peptide.

In one embodiment the peptide is provided to the APC in the absence of the T cell. The APC is then provided to the T cell, typically after being allowed to present the peptide on its surface. The peptide may have been taken up inside the APC and presented, or simply be taken up onto the surface without entering inside the APC.

The duration for which the peptide is contacted with the T cells will vary depending on the method used for determining recognition of the peptide. Typically 10^5 to 10^7, preferably $5x10^5$ to 10^6 PBMCs are added to each assay. In the case where peptide is added directly to the assay its concentration is from 10^{-1} to $10^3\mu g/ml$, preferably 0.5 to 50μg/ml or 1 to 10μg/ml.

Typically the length of time for which the T cells are incubated with the peptide is from 4 to 24 hours (preferably 6 to 16 hours) for effector T cells or for more than 24 hours for central memory cells. When using *ex vivo* PBMCs it has been found that $0.3x10^6$ PBMCs can be incubated in 10μg/ml of peptide for 12 hours at 37˚C.

The method may be based on an ELISA method, such as the whole blood Quantiferon system and its modifications (for example as available from Cellestis).

The determination of the recognition of the peptide by the T cells may be done by measuring the binding of the peptide to the T cells. Typically T cells which bind the peptide can be sorted based on this binding, for example using a FACS machine. The presence of T cells which recognise the peptide will be deemed to occur if the frequency of cells sorted using the peptide is above a 'control' value. The frequency of antigen-experienced T cells is generally 1 in 10^6 to 1 in 10^3, and therefore whether or not the sorted cells are antigen-experienced T cells can be determined.

The determination of the recognition of the peptide by the T cells may be measured *in vivo*. Typically the peptide is administered to the host and then a response which indicates recognition of the peptide may be measured. In one embodiment the peptide is administered intradermally, typically in a similar manner to the Mantoux test. The peptide may be administered epidermally. The peptide is typically administered by needle, such as by injection, but can be administered by other methods such as ballistics, for example the ballistics techniques which have been used to deliver nucleic acids. EP-A-0693119 describes techniques which can typically be used to administer the peptide. Typically from 0.001 to 1000 μg, for example from 0.01 to 100 μg or 0.1 to 10 μg of peptide is administered.

Alternatively an agent can be administered which is capable of providing the peptides *in vivo*. Thus a polynucleotide capable of expressing the peptide can be administered, typically in any of the ways described above for the administration of the peptide. The polynucleotide typically has any of the characteristics of the polynucleotide provided by the invention which is discussed below. Peptide is expressed from the polynucleotide *in vivo* and recognition of the peptide *in vivo* is measured. Typically from

0.001 to 1000 μg, for example from 0.01 to 100 μg or 0.1 to 10 μg of polynucleotide is administered.

Recognition of the peptide *in vivo* is typically indicated by the occurrence of a DTH response. This is generally measured by visual examination of the site of administration of the peptide to determine the presence of inflammation, such as by the presence of induration, erythema or oedema.

The peptide capable of binding to a T-cell receptor which recognises a peptide having the sequence shown in SEQ ID NO:1 or any other peptides to be tested (i.e. analogues of the peptide) may be identified by any suitable method. The binding of the peptide to the said T cell receptors can be tested by standard techniques. For example, T cell receptors can be isolated from T cells which have been shown to recognise the peptide having a sequence shown in SEQ ID NO:1 (e.g. using the method of the invention). Demonstration of the binding of the peptide to the T cell receptors can then shown by determining whether the T cell receptors inhibit the binding of the peptide to a substance that binds the peptide, e.g. an antibody to the peptide. Typically the peptide is bound in an MHC molecule in such an inhibition of binding assay.

Typically the analogue inhibits the binding of the peptide to a T cell receptor. In this case the amount of peptide which can bind the T cell receptor in the presence of the analogue is decreased. This is because the analogue is able to bind the T cell receptor and therefore competes with the peptide for binding to the T cell receptor.

T cells for use in the above binding experiments can be isolated from patients with mycobacterial infection, for example with the aid of the method of the invention.

The analogue may have homology with the equivalent original peptide represented by one of SEQ ID NO:1 or a sequence of at least 8 consecutive amino acids of SEQ ID NO:1. A peptide which is homologous to another peptide is typically at least 70% homologous to the peptide, preferably at least 80 or 90% and more preferably at least 95%, 97% or 99% homologous thereto, for example over a region of at least 8, at least 15, preferably at least 30, for instance at least 40, 60 or 100 or more contiguous amino acids. Methods of measuring protein homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is calculated on

the basis of amino acid identity (sometimes referred to as "hard homology"). For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* **12**, p387-395).

Typically the amino acids in the analogue at the equivalent positions to amino acids in the original peptide which contribute to binding the MHC molecule or are responsible for the recognition by the T cell receptor, are the same or are conserved.

Typically the analogue comprises one or more modifications, which may be natural post-translation modifications or artificial modifications. The modification may provide a chemical moiety (typically by substitution of a hydrogen, e.g. of a C-H bond), such as an amino, acetyl, hydroxy or halogen (e.g. fluorine) group or carbohydrate group. Typically the modification is present on the N or C terminus.

The peptide may comprise one or more non-natural amino acids, for example amino acids with a side chain different from natural amino acids. Generally, the non-natural amino acid will have an N terminus and/or a C terminus. The non-natural amino acid may be an L-amino acid.

The peptide typically has a shape, size, flexibility or electronic configuration which is substantially similar to the original peptide. It is typically a derivative of the original peptide.

In one embodiment the peptide is or mimics the original peptide bound to a MHC class II molecule. The analogue may be or may mimic the original peptide bound to 2, 3, 4 or more MHC class II molecules associated or bound to each other. These MHC molecules may be bound together using a biotin/streptavidin based system, in which typically 2, 3 or 4 biotin labelled MHC molecules bind to a streptavidin moiety. This peptide typically inhibits the binding of the peptide/MHC Class II complex to a T cell receptor or antibody which is specific for the complex. The analogue may be an antibody or a fragment of an antibody, such as a Fab or $(Fab)_2$ fragment.

The peptide may be immobilised on a solid support.

The peptide is typically designed by computational means and then synthesised using methods known in the art. Alternatively it can be selected from a library of

compounds. The library may be a combinatorial library or a display library, such as a phage display library. The library of compounds may be expressed in the display library in the form of being bound to a MHC class II molecule, such as the MHC molecule which the original peptide binds. Peptides are generally selected from the library based on their ability to mimic the binding characteristics of the original peptides. Thus they may be selected based on ability to bind a T cell receptor or antibody which recognises the original peptide.

The invention also provides a kit for carrying out the method comprising one or more of the peptides and a means to detect the recognition of the peptide by the T cell. Typically the peptides are provided for simultaneous, separate or sequential use. Typically the means to detect recognition allows or aids detection based on the techniques discussed above.

Thus the means may allow detection of a substance secreted by the T cells after recognition. The kit may thus additionally include a specific binding agent for the substance, such as an antibody. The agent is typically specific for IFN-γ. The agent is typically immobilised on a solid support. This means that after binding the agent the substance will remain in the vicinity of the T cell which secreted it. Thus 'spots' of substance/agent complex are formed on the support, each spot representing a T cell which is secreting the substance. Quantifying the spots, and typically comparing against a control, allows determination of recognition of the peptide.

The kit may also comprise a means to detect the substance/agent complex. A detectable change may occur in the agent itself after binding the substance, such as a colour change. Alternatively a second agent directly or indirectly labelled for detection may be allowed to bind the substance/agent complex to allow the determination of the spots. As discussed above the second agent may be specific for the substance, but binds a different site on the substance than the first agent.

The immobilised support may be a plate with wells, such as a microtitre plate. Each assay can therefore be carried out in a separate well in the plate.

The kit may additionally comprise medium for the T cells, detection agents or washing buffers to be used in the detection steps. The kit may additionally comprise

reagents suitable for the separation from the sample, such as the separation of PBMCs or T cells from the sample. The kit may be designed to allow detection of the T cells directly in the sample without requiring any separation of the components of the sample.

The kit may comprise an instrument which allows administration of the peptide, such as intradermal or epidermal administration. Typically such an instrument comprises one or more needles. The instrument may allow ballistic delivery of the peptide. The peptide in the kit may be in the form of a pharmaceutical composition.

The kit may also comprise controls, such as positive or negative controls. The positive control may allow the detection system to be tested. Thus the positive control typically mimics recognition of the peptide in any of the above methods. Typically in the kits designed to determine recognition in vitro the positive control is a cytokine. In the kit designed to detect *in vivo* recognition of the peptide the positive control may be antigen to which most individuals should response.

The kit may also comprise a means to take a sample containing T cells from the human, such as a blood sample. The kit may comprise a means to separate mononuclear cells or T cells from a sample from the human.

The invention also provides a composition comprising a peptide of the invention. The composition may be a pharmaceutical composition which further comprises a pharmaceutically acceptable carrier or diluent. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. Typically the composition is formulated for intradermal or epidermal administration or for application by ballistic techniques. Thus the peptide or polynucleotide may be associated with a carrier particle for ballistic delivery.

The invention also relates to a polynucleotide which is capable of expressing one or more peptides of the invention. Typically the polynucleotide is DNA or RNA, and is single or double stranded. The polynucleotide therefore typically comprises sequence which encodes the sequence of SEQ ID NO: 1 or a fragment thereof.

5' and/or 3' to the sequence encoding the peptide the polynucleotide has coding or non-coding sequence. Sequence 5' and/or 3' to the coding sequence may comprise sequences which aid expression, such as transcription and/or translation, of the sequence

encoding the peptide. The polynucleotide may be capable of expressing the peptide in a prokaryotic or eukaryotic cell. In one embodiment the polynucleotide is capable of expressing the peptide in a mammalian cell, such as a human, primate or rodent cell.

The polynucleotide may be incorporated into a replicable vector. Such a vector is able to replicate in a suitable cell. The vector may be an expression vector. In such a vector the polynucleotide of the invention is operably linked to a control sequence which is capable of providing for the expression of the polynucleotide. The vector may contain a selectable marker, such as the ampicillin resistance gene.

The polynucleotide of the invention, the peptides in a composition of the invention or the agents used in the method (for example in the detection of substances secreted from T cells) may carry a detectable label. Detectable labels which allow detection of the secreted substance by visual inspection, optionally with the aid of an optical magnifying means, are preferred. Such a system is typically based on an enzyme label which causes colour change in a substrate, for example alkaline phosphatase causing a colour change in a substrate. Such substrates are commercially available, e.g. from BioRad. Other suitable labels include other enzymes such as peroxidase, or protein labels, such as biotin; or radioisotopes, such as ^{32}P or ^{35}S. The above labels may be detected using known techniques.

Polynucleotides of the invention or peptides in a composition of the invention may be in substantially purified form. They may be in substantially isolated form, in which case they will generally comprise at least 90%, for example at least 95, 97 or 99% of the polynucleotide, peptide or antibody in the preparation. The substantially isolated peptides generally comprise at least 90%, such as for example at least 95, 97 or 99% of the dry mass of the preparation. The polynucleotide or peptide are typically substantially free of other cellular components or substantially free of other mycobacterial cellular components. The polynucleotide or peptide may be used in such a substantially isolated, purified or free form in the method or be present in such forms in the kit.

The peptide for use in the invention can be made using standard synthetic chemistry techniques, such as by use of an automated synthesizer.

The peptide is typically made from a longer polypeptide e.g. a fusion protein, which polypeptide typically comprises the sequence of the peptide. The peptide may be derived from the polypeptide by for example hydrolysing the polypeptide, such as using a protease; or by physically breaking the polypeptide. The polypeptide is typically has the sequence shown in SEQ ID NO:1 and may have been expressed recombinantly.

The peptide can also be made in a process comprising expression of a polynucleotide, such as by expression of the polynucleotide of the invention. The expressed polypeptide may be further processed to produce the peptide of the invention. Thus the peptide may be made in a process comprising cultivating a cell transformed or transfected with an expression vector as described above under conditions to provide for expression of the peptide or a polypeptide from which the peptide can be made. The polynucleotide of the invention can be made using standard techniques, such as by using a synthesiser.

The invention also provides a method of ascertaining the stage of a *Mycobacterium tuberculosis* infection in a human comprising determining whether there is a differential T cell response to different MTB antigens in the human. Any suitable method mentioned herein may be used to measure the T cell responses. The T cell responses may be to any of the MTB antigens mentioned herein, such as one or more of Rv3879c, ESAT-6, CFP10, Rv3873, Rv3878, Rv1989c. The method may be carried out to determine whether the infection is recent or longstanding, to determine whether the human is latently infected or has disease, or to monitor the effect of treatment.

The invention is illustrated by the following Examples:

Example 1

Study participants

All participants were recruited prospectively in London and Oxford over a 14 month period from June 2002 through July 2003. Ethical approval for the study was granted by the Harrow and Central Oxford Research Ethics Committees. The diagnoses of all 49 TB patients were bacteriologically confirmed with positive cultures for MTB from one or more clinical specimens. Patients were untreated or had received less than 2 weeks

therapy at the time of venepuncture for ELISPOT assay. Control participants were healthy BCG-vaccinated laboratory personnel from regions with a low prevalence of TB and with no known exposure to MTB. All had recently tested negative by IFN-γ-ELISPOT using 38 overlapping 15-mer peptides spanning the length of ESAT-6 and CFP10, as previously described (Lalvani *et al.* 1997. J. Exp. Med. 186:859-865).

Epidemiological data regarding place of birth, any period of residence in higher prevalence regions and absence of TB contact was collected from these volunteers at the point of venepuncture. Health care workers were not recruited due to the risk of occupational TB exposure.

Peptides

Sixty-seven synthetic peptides spanning selected regions of four open reading frames (ORFs) were designed and purchased (Research Genetics, Huntsville, AL, USA). The peptides were selected from those used in (Cockle *et al.* 2002 Infect. Immunol.. 70:6996-7003). The Rv3879c peptides are 15mer peptides overlapping by 10 amino acids which represent 95 out of 729 amino acids of the Rv3879c primary amino acid sequence. This selection of peptides represents only 13% of the entire sequence of Rv3879c.

In the case of all four molecules except Rv3873, these sequences were at the amino terminus, and the exact regions represented by the peptides for each molecule are shown in table 2. Each peptide was 15 residues long and overlapped the adjacent peptide by 10 amino acids (a.a.). This approach has previously been shown to be effective for detecting HLA class I-restricted CD8 as well as HLA class II-restricted CD4 T cell responses (Pathan *et al.* 2000. Eur. J. Immunol. 30:2713-2721). The 67 peptides were arranged into 11 pools containing between five and seven peptides and Table 2 shows the pools in relation to the antigens they represent. For all peptides, identity was confirmed by mass spectrometry and purity was more than 70%.

***Ex vivo* IFN-γ ELISPOT assays**

ELISPOT assays were performed as previously described (Lalvani *et al.* 1997. J. Exp. Med. 186:859-865 Lalvani *et al.* 2001. Am. J. Respir. Crit. Care. Med. 163: 824-828). IFN-γ-ELISPOT plates (Mabtech AB, Stockholm, Sweden), were seeded with 2.5×10^5 PBMCs per well: duplicate wells contained no antigen (negative control), phytohaemagglutinin (PHA, positive control, ICN Biomedical OH, USA), at 5μg/ml, streptokinase/streptodornase (SKSD, Varidase, Cyanamid, Hampshire, UK) at 100u/ml, Purified Protein Derivative (PPD, Statens Serum Institut, Denmark) at 20μg/ml, and one of 11 peptide pools, such that the final concentration of each peptide was 10μg/ml. After overnight incubation at 37°C, 5% CO_2, the plates were developed with preconjugated detector antibody and chromogenic substrate, 5-bromo-4-chloro-3-indolyl-phosphate p-nitro blue tetrazolium chloride (BCIP/NBT plus, Moss Inc, Pasadena, MD, USA). For unexposed BCG-vaccinated donors who responded to any of the pools, PBMC were retested against all 67 peptides individually in single ELISPOT wells at a final concentration of 10μg/ml.

Assays were scored by an automated ELISPOT counter (AID-GmbH, Strassberg, Germany). For wells containing peptide pools, responses were scored as positive if the test well contained at least five more IFN-γ spot-forming cells (SFC) than negative control wells and this number also had to be at least twice the frequency found in the negative control wells. These pre-defined cut-off points translate into a detection threshold of 20 peptide-specific T cells per million PBMC. The person performing the assays was blind to personal identifiers of participants.

Bioinformatics

The DNA sequence of MTB H37Rv was visualized using the TubercuList database (http://genolist.pasteur.fr/TubercuList/). Basic Local Alignment Search Tool (BLAST) searches for protein sequence homology in available mycobacterial genomes were performed using TubercuList, the Sanger Centre server (Cambridge, UK) for the incomplete *M. bovis BCG* genome sequence

(http://www.sanger.ac.uk/Projects/M_bovis/) and the National Center for Biotechnology Information BLAST server (http://www.ncbi.nlm.nih.gov/BLAST).

Example 2

Demographic characteristics of study participants

Demographic characteristics of the 49 culture-confirmed TB patients are shown in Table 1. 42 patients had pulmonary TB, of whom 23 were sputum smear-positive. The 7 patients with extra-pulmonary TB, comprised patients with pleural TB (n=3); lymphadenitis (n=1), miliary TB (n=2) and urinary tract TB (n= 1). The patients were from a broad range of ethnicities. Demographic characteristics of BCG donors are shown in Table 1. All donors were born in regions of low prevalence for TB (Europe or Australia). None had a history of known TB contact and none had resided for more then 3 months in high prevalence regions.

Example 3

IFN-γ ELISPOT responses to peptides from Rv3873, Rv3878, Rv3879c and Rv1989c in culture-confirmed tuberculosis patients

IFN-γ ELISPOT responses of PBMC from all 49 TB patients to the 11 peptide pools from the four antigens are summarized in Fig 1A. The percentages of responding patients varied between 25.5% and 53.1% for the different antigens (Fig 1B). The proportion of patients responding to peptides from each of the antigens Rv3873, Rv3879c, Rv3878 and Rv1989c was 53.1% (95% CI 39-67%), 44.7% (95% CI 31-57%), 34.7% (95% CI 22-48%) and 25.5% (95% CI 13-39%), respectively (Fig. 1B). Combining these responses, 30 of 49 tuberculosis patients responded to peptide pools from one or more antigens, giving a diagnostic sensitivity of 61.2% (95% confidence interval [CI] 46.2%-74.8%) for all peptides used together. This contrasts with the results obtained by Cockle *et al*, who found that peptides from Rv3873, Rv3878, Rv3879c, Rv1989c could together be used to detect almost all infected cattle.

The frequencies of Rv3873, Rv3878, Rv3879c, Rv1989c peptides-specific IFN-γ-secreting T cells for all responder patients were (median response and inter quartile

ranges[IQR]): 115 [52 to 310], 112 [72 to 128], 82 [28 to 116], and 76 [45 to 296] per million PBMCs, respectively (Fig. 2A).

Importantly, 3 of the 49 TB patients who responded to Rv3879c peptides failed to respond to any of 35 15mer peptides representing the entire sequences of ESAT-6 and CFP10.

Example 4

Comparison of proportions of patients responding to each antigen according to the clinical type of TB

The TB patients were stratified by clinical type of TB, i.e. pulmonary (n=42) versus extra-pulmonary (n=7) TB. The proportion of patients from each group that responded to peptides from each different protein were then compared. Although there was no significant difference between the proportion of pulmonary and extra-pulmonary patients that responded to Rv3873, Rv3878 and Rv1989c, significantly more extra-pulmonary patients (6/7, 86%) responded to Rv3879c than did pulmonary patients (14/42, 33%), (p=0.014).

Example 5

IFN-γ ELISPOT responses in BCG-vaccinated healthy donors

Rv3873 peptide pools elicited responses in 3/38 (7.9%) BCG-vaccinated unexposed donors; Rv3878 and Rv3879c each elicited a response in one (2.6%) donor; and Rv1989c elicited no responses. Two donors, donors 20 and 31, each responded to a different peptide from pool 2 of Rv3873, and one, donor 25, responded to pools from the Rv3873, Rv3878 and Rv3879c (Table 2 and Fig 1). Donors 20 and 31 responded to peptides 119-133 (LTATNFFGINTIPIA) and 139-153 (YFIRMWNQAALAMEV), respectively, both from pool 2 of Rv3873. Donor 25 responded to peptide 174-188 (LDPGASQSTTNPIFG) from Rv3873, peptides 16-30 (AAKLAGLVFPQPPAP) and 61-75 (ESLVSDGLPGVKAAL) from Rv3878 and 26-40 (DTFYDRAQEYSQVLQ) from Rv3879c. Combining all these responses, 3 of 38 (7.9%) BCG vaccinated healthy donors responded to one or more antigens, while 81.6% responded to PPD.

The frequencies of peptide specific IFN-γ SFCs seen in BCG-vaccinated unexposed donors were much lower than in the TB patients (Fig 2B). The median frequencies of peptide-specific T cells (and inter-quartile range) were: 28 (24 to 56), 72 (72) and 20 (20) per million PBMC for Rv3873, Rv3878 and Rv3879c respectively (Fig 2A).

Example 6

BLAST searches of cross-reactive peptide sequences

BLAST searches for protein sequences highly homologous to the six 15mer peptides that gave a response in BCG-vaccinated donors were performed. Peptide 119-133 (LTATNFFGINTIPIA), had the greatest homology with 93% identity to other mycobacterial proteins (14 out of 15 amino acids identical). This peptide is from pool 2 of Rv3873, a member of the PPE family of proteins, and is encoded within a 52 a.a. long motif that is highly conserved throughout the PPE family (Fig. 3). Consequently it displays high levels of homology with many MTB, *M. bovis* and *M. leprae* PPE proteins (Table 3) that are encoded in the deleted and undeleted regions of the genomes of MTB, *M. bovis* and other mycobacteria. Peptide 139-153 (YFIRMWNQAALAMEV), which is also encoded within the 52 a.a. conserved motif of Rv3873 (Fig. 3), also showed homology with sequences from many PPE proteins (Table 3) although the level of identity was considerably lower at 47% (7 out of 15 identical residues). In contrast, peptide 174-188 (LDPGASQSTTNPIFG) from Rv3873, which lies outside the conserved motif region, had no significant homology with PPE family members. The two cross-reactive peptides from Rv3878 and the single cross-reactive peptide from Rv3879c, had no significant sequence homology with any other mycobacterial proteins.

Discussion

We have evaluated human cellular immune responses to peptide mixtures of four MTB proteins encoded in regions of difference RD1 and RD2. This is the first such report for Rv3879c and Rv1989c; for Rv3873 and Rv3878 cellular immune responses were also recently described by Okkels et al (2003. Infect. Immun. 71: 6116-23). Peptides from

each protein were recognized by T cells from >25% of TB patients in IFN-γ-ELISPOT assays. Peptide pools from two RD1-encoded gene products were recognized in approximately half of all TB patients tested: Rv3879c (45%) and the PPE family member Rv3873 (53%). This study thus identifies these two proteins as major MTB T cell antigens in infected humans. IFN-γ-ELISPOT responses to the peptides were rare in BCG-vaccinated donors, giving a specificity of 97.4% or more for all antigens except Rv3873, which, on account of cross-reactive peptides from conserved sequences, had a lower specificity of 92.1%. The high specificity of the Rv3879c peptides (97.4%), together with the fact that they are recognized in IFN-γ-ELISPOT by almost a half of TB patients, identifies this molecule as a useful T cell antigen for inclusion in novel T cell-based diagnostic tests of MTB infection.

CMI to the antigens in this study has previously been assessed in cattle (Cockle *et al.* 2002). Despite being encoded in RD1, peptides derived from Rv3873 and Rv3879c elicited IFN-γ responses in a whole blood ELISA assay in 17% and 33% of BCG-vaccinated cattle respectively. However, the responses were only borderline positive, and the number of vaccinated cattle tested was low (n=6). In our larger series of BCG-vaccinated humans, we have shown that the level of cross-reactivity of these antigens with BCG is far lower than in cattle. Moreover, 3 of the 5 responses observed were borderline positive (Fig. 2B).

T cell responses to peptides spanning the length of ESAT-6/CFP10 have been detected in 70-80% of TB patients using IFN-γ ELISA and around 90% of TB patients using IFN-γ-ELISPOT assay. We have shown that T cell responses to Rv3879c peptides also occur in MTB infected humans, and further that some culture confirmed TB patients who failed to respond to ESAT-6 and CFP10 peptides did respond Rv3879c peptides. Thus Rv3879c peptides may be used to further enhance the sensitivity of T cell-based assays using ESAT-6/CFP10, without compromising specificity.

TABLE 1. Demographic characteristics of TB patients and unexposed BCG-vaccinated donors

	Patients with tuberculosis (n=49) (%)	**BCG donors (n=38) (%)**
Mean age in years(range)	34.0±13.4(17-78)	33.3±6.7(20-50)
Sex (male/female)	31/18(63/37)	22/16(58/42)
Ethnicity		
Indian Sub-Continent	24(49)	1
African	18(37)	0
Oriental	4(8)	0
White	3(6)	37

TABLE 2. Antigens and peptide pools evaluated and number of donors who responded to each peptide pool by IFN-γ-ELISPOT assay

Region of Difference	Designation	Size (amino acids)	Putative Function[c]	Peptide Pools (no. of constituent peptides)	Region of molecule represented by peptide pools (aa position)	No. TB patients responding n=49	No. unexposed BCG vaccinated donors responding n=38
RD1	Rv 3873	368	Member of the M. TB PPE family	Pool 1 (6)	89-128	8	0
				Pool 2 (6)	129-158	25	2
				Pool 3 (6)	159-188	18	1
	Rv 3878	280	Unknown alanine rich protein	Pool 1 (7)	1-45	16	1
				Pool 2 (7)	36-80	14	1
	Rv3879c	729	Unknown alanine and proline rich protein	Pool 1 (6)	1-40	12[a]	1
				Pool 2 (6)	31-70	18[a]	0
				Pool 3 (5)	61-95	9[b]	0
RD2	Rv 1989c	186	Unknown	Pool 1 (6)	1-40	10[b]	0
				Pool 2 (6)	31-70	7[b]	0
				Pool 3 (6)	61-100	8[b]	0

[a]n=48

[b]n=47

[c]Putative function as suggested by Cole *et al.* 1998. Nature 393:537-544

TABLE 3. Homology between peptides 119-133 and 139-153 from Rv3873 with sequences from other mycobacterial proteins.

(i) Peptide 119-133

Designation[a]	**Putative Function**	**Amino Acid Sequence**[b]
Rv3873	***M. tuberculosis* PPE family**	**LTATNFFGINTIPIA**
Rv3021c,3018c,0280,1387	*M. tuberculosis* PPE family	LVATNFFGINTIPIA
Rv0256c	*M. tuberculosis* PPE family	LMATNFFGINTIPIA
Rv0453	*M. tuberculosis* PPE family	MVATNFFGINTIPIA

(ii) Peptide 139-153

Designation[a]	**Putative Function**	**Amino Acid Sequence**[b]
Rv3873	***M. tuberculosis* PPE family**	**YFIRMWNQAALAMEV**
Rv2768c,1039c	*M. tuberculosis* PPE family	HYGEMWAQDALAMYG
Rv0286	*M. tuberculosis* PPE family	DYVRMWLQAAAVMGL
Rv1807	*M. tuberculosis* PPE family	QYAEMWSQDAMAMYG

The homology search was performed using the BLAST program.

[a]Designation of *M. tuberculosis* proteins as described(18). Sequences of all related proteins described are also present in the *M. bovis* BCG genome (http://www.sanger.ac.uk/Projects/M_bovis/). Non-identical residues are underlined.

[b]Amino acid residues are shown in the one letter code.

SEQ ID NO:1 - Rv3879c

MSITRPTGSYARQMLDPGGWVEADEDTFYDRAQEYSQVLQRVTDVLDTCRQQKGHVFEGG
LWSGGAANAANGALGANINQLMTLQDYLATVITWHRHIAGLIEQAKSDIGNNVDGAQREI
DILENDPSLDADERHTAINSLVTATHGANVSLVAETAERVLESKNWKPPKNALEDLLQQK
SPPPPDVPTLVVPSPGTPGTPGTPITPGTPITPGTPITPIPGAPVTPITPTPGTPVTPVT
PGKPVTPVTPVKPGTPGEPTPITPVTPPVAPATPATPATPVTPAPAPHPQPAPAPAPSPG
PQPVTPATPGPSGPATPGTPGGEPAPHVKPAALAEQPGVPGQHAGGGTQSGPAHADESAA
SVTPAAASGVPGARAAAAAPSGTAVGAGARSSVGTAAASGAGSHAATGRAPVATSDKAAA
PSTRAASARTAPPARPPSTDHIDKPDRSESADDGTPVSMIPVSAARAARDAATAAASARQ
RGRGDALRLARRIAAALNASDNNAGDYGFFWITAVTTDGSIVVANSYGLAYIPDGMELPN
KVYLASADHAIPVDEIARCATYPVLAVQAWAAFHDMTLRAVIGTAEQLASSDPGVAKIVL
EPDDIPESGKMTGRSRLEVVDPSAAAQLADTTDQRLLDLLPPAPVDVNPPGDERHMLWFE
LMKPMTSTATGREAAHLRAFRAYAAHSQEIALHQAHTATDAAVQRVAVADWLYWQYVTGL LDRALAAAC

SEQ ID NO's 2 to 18 - Rv3879c peptides mentioned in Table 2

POOL 1

2 MSITR PTGSY ARQML

3 PTGSY ARQML DPGGW

4 ARQML DPGGW VEADE

5 DPGGW VEADE DTFYD

6 VEADE DTFYD RAQEY

7 DTFYD RAQEY SQVLQ

POOL 2

8 RAQEY SQVLQ RVTDV

9 SQVLQ RVTDV LDTCR

10 RVTDV LDTCR QQKGH

11 LDTCR QQKGH VFEGG

12 QQKGH VFEGG LWSGG

13 VFEGG LWSGG AANAA

POOL 3

14 LWSGG AANAA NGALG

15 AANAA NGALG ANINQ

16 NGALG ANINQ LMTLQ

17 ANINQ LMTLQ DYLAT

18 LMTLQ DYLAT VITWH

ESAT-6

MTEQQWNFAGIEAAASAIQGNVTSIHSLLDEGKQSLTKLAAAWGGSGSEAYQGVQQKWDA
TATELNNALQNLARTISEAGQAMASTEGNVTGMFA

CFP10

MAEMKTDAATLAQEAGNFERISGDLKTQIDQVESTAGSLQGQWRGAAGTAAQAAVVRFQE
AANKQKQELDEISTNIRQAGVQYSRADEEQQQALSSQMGF

CLAIMS

1. A method of diagnosing *Mycobacterium tuberculosis* infection in a human, or of determining whether a human has been exposed to *Mycobacterium tuberculosis*, comprising:

(i) contacting T-cells from said human with one or more of

(a) a peptide having the sequence shown in SEQ ID NO: 1;

(b) a peptide having or comprising the sequence of at least 8 consecutive amino acids of the sequence shown in SEQ ID NO: 1; or

(c) a peptide having or comprising a sequence which is capable of binding to a T-cell receptor which recognises a peptide as defined in (a) or (b); and

(ii) determining whether any of the said T-cells recognise said peptide,

wherein steps (i) and (ii) are optionally carried out *in vitro*.

2. A method of increasing the sensitivity of a diagnostic test for diagnosing *Mycobacterium tuberculosis* infection in a human, wherein said diagnostic test comprises contacting T cells from said human with *a Mycobacterium tuberculosis* antigen which is not Rv3879c, said method additionally comprising

(i) contacting T-cells from said human with one or more of

(a) a peptide having the sequence shown in SEQ ID NO: 1;

(b) a peptide having or comprising the sequence of at least 8 consecutive amino acids of the sequence shown in SEQ ID NO: 1; or

(c) a peptide having or comprising a sequence which is capable of binding to a T-cell receptor which recognises a peptide as defined in (a) or (b); and

(ii) determining whether any of the said T-cells recognise said peptide,

wherein steps (i) and (ii) are optionally carried out *in vitro*.

3. A method according to claim 1 or 2, wherein step (i) further comprises contacting

said T-cells with one or more further *Mycobacterium tuberculosis* T-cell antigen(s) or with an analogue(s) of said antigen(s) which is capable of binding to a T-cell receptor which recognises said antigen(s).

4. A method according to claim 3, wherein said one or more further T-cell antigens include antigens encoded by the RD-1 or RD-2 region, which antigens are preferably ESAT-6 and/or CFP10; or fragments thereof which are at least 8 amino acids long.

5. A method according to any one of claims 2 to 4, wherein said one or more further T-cell antigens include Rv3873, Rv3878 or Rv1989c; or fragments thereof which are at least 8 amino acids long.

6. A method according to any one of the preceding claims, wherein step (i) comprises contacting said sample of T-cells with two or more different peptides, each having the sequence of at least 8 consecutive amino acids of the sequence shown in SEQ ID NO: 1.

7. A method according to any one of the preceding claims wherein peptides from, or analogues of, at least five different antigens are contacted with the T cells.

8. A method according to any one of the preceding claims wherein one or more of the peptides

(i) represented by SEQ ID NO's 2 to 18, or

(ii) which bind to a T-cell which recognise (i),

are contacted with the T cells.

9. A method according to any one of the preceding claims, wherein recognition of said peptide by said T-cells is determined by detecting the secretion of a cytokine from the T-cells.

10. A method according to claim 9, wherein the cytokine is IFN-γ.

11. A method according to claim 9 or 10, wherein said cytokine is detected by allowing said cytokine to bind to an immobilised antibody specific to said cytokine and detecting the presence of the antibody/cytokine complex.

12. A method according to any one of the preceding claims, wherein said T-cells are freshly isolated *ex vivo* cells.

13. A method according to any one of claims 1 to 11, wherein said T-cells have been cultured *in vitro*.

14. Use of

(i) a peptide as defined in claim 1 or 8, and optionally also an antigen as defined in any one of claims 3 to 5, or

(ii) a polynucleotide which is capable of expressing (i),

in the manufacture of a diagnostic means for diagnosing *Mycobacterium tuberculosis* infection or exposure in a human.

15. A diagnostic composition comprising a peptide as defined in claim 1 or 8 and optionally one or more further *Mycobacterium tuberculosis* T-cell antigens.

16. A composition according to claim 15 wherein said one or more further T-cell antigens are selected from

(i) ESAT-6, CFP10, Rv3873, Rv3878, Rv1989c or fragment of any thereof which is at least 8 amino acids long; or

(ii) an analogue of (i) which binds to a T-cell which recognises (i).

17. A kit for diagnosing *Mycobacterium tuberculosis* infection or exposure in a human, comprising one or more peptides as defined in claim 1 or 8 or a composition according to claim 15 or 16, and optionally a means for detecting recognition of a peptide by T-cells.

18. A kit according to claim 17, wherein said means for detecting recognition of a peptide by T-cells comprises an antibody to a cytokine.

19. A kit according to claim 18, wherein said antibody is immobilised on a solid support and wherein said kit optionally comprises a means to detect an antibody/cytokine complex.

20. A kit according to claim 18 or 19, wherein said cytokine is IFN-γ.

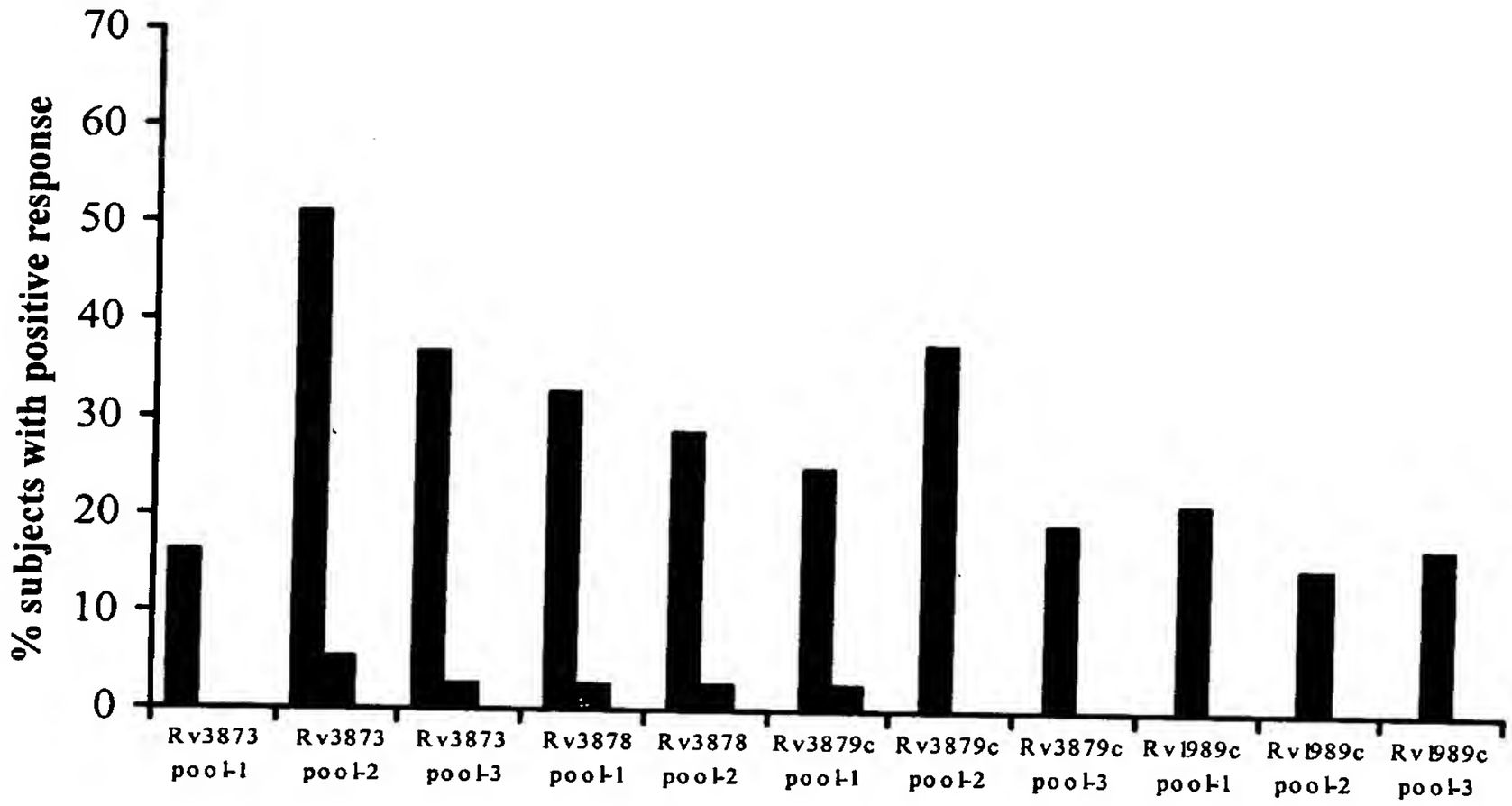
21. A method of ascertaining the stage of a *Mycobacterium tuberculosis* infection in a human comprising determining whether there is a differential T cell response to different antigens in the human.

22. A method according to claim 21 wherein T cell responses to one or more of Rv3879c, ESAT-6, CFP10, Rv3873, Rv3878, Rv1989c are measured.

23. A method according to claim 21 or 22 which is carried out to
(i) to determine whether the infection is recent or longstanding, or
(ii) to determine whether the human is latently infected or has disease, or
(iii) to monitor the effect of treatment.

Figure 1

A.


% subjects with positive response
70
60
50
40
30
20
10
0
Rv3873 pool-1
Rv3873 pool-2
Rv3873 pool-3
Rv3878 pool-1
Rv3878 pool-2
Rv3879c pool-1
Rv3879c pool-2
Rv3879c pool-3
Rv1989c pool-1
Rv1989c pool-2
Rv1989c pool-3
Peptide Pools

B.

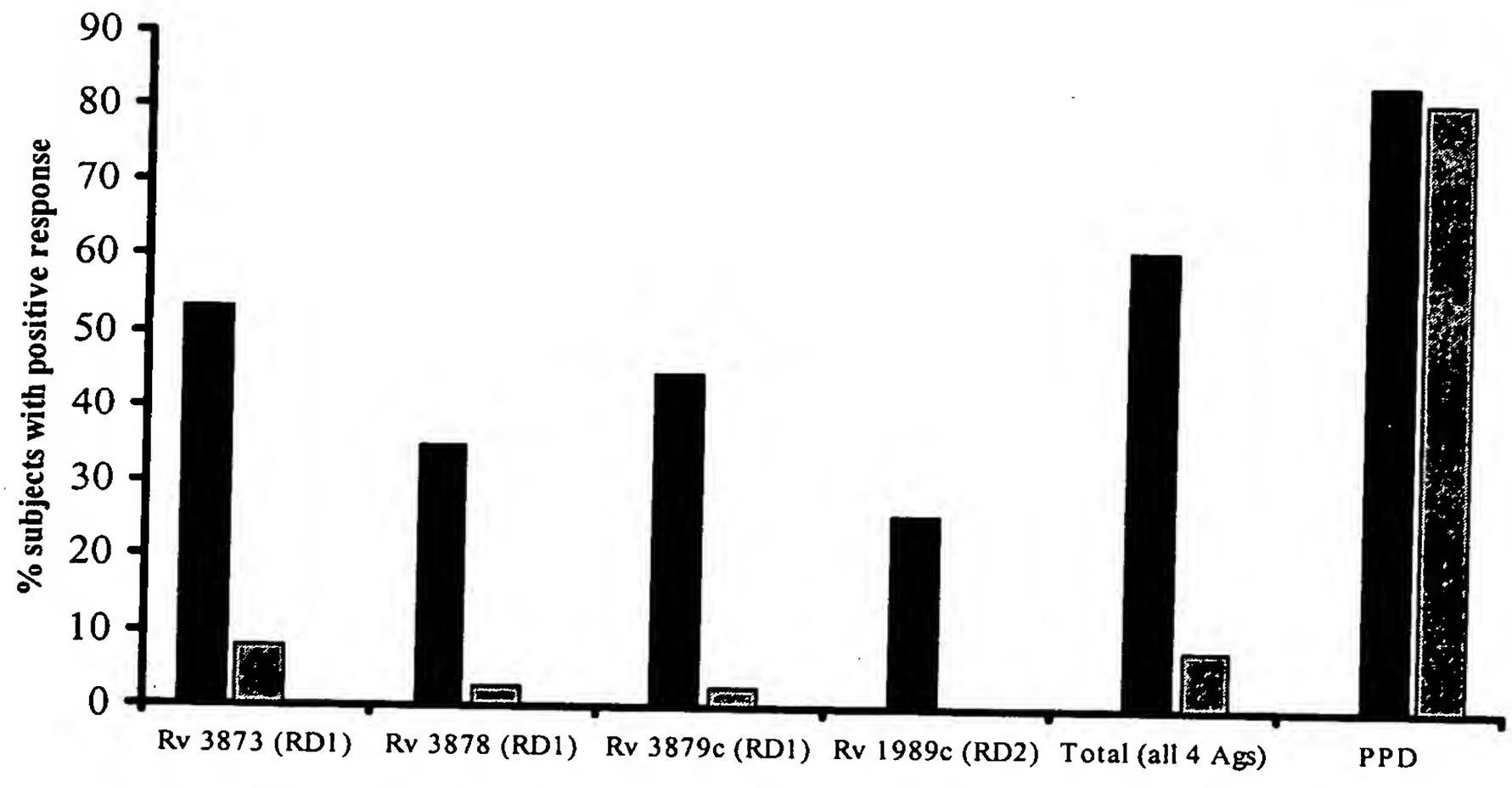
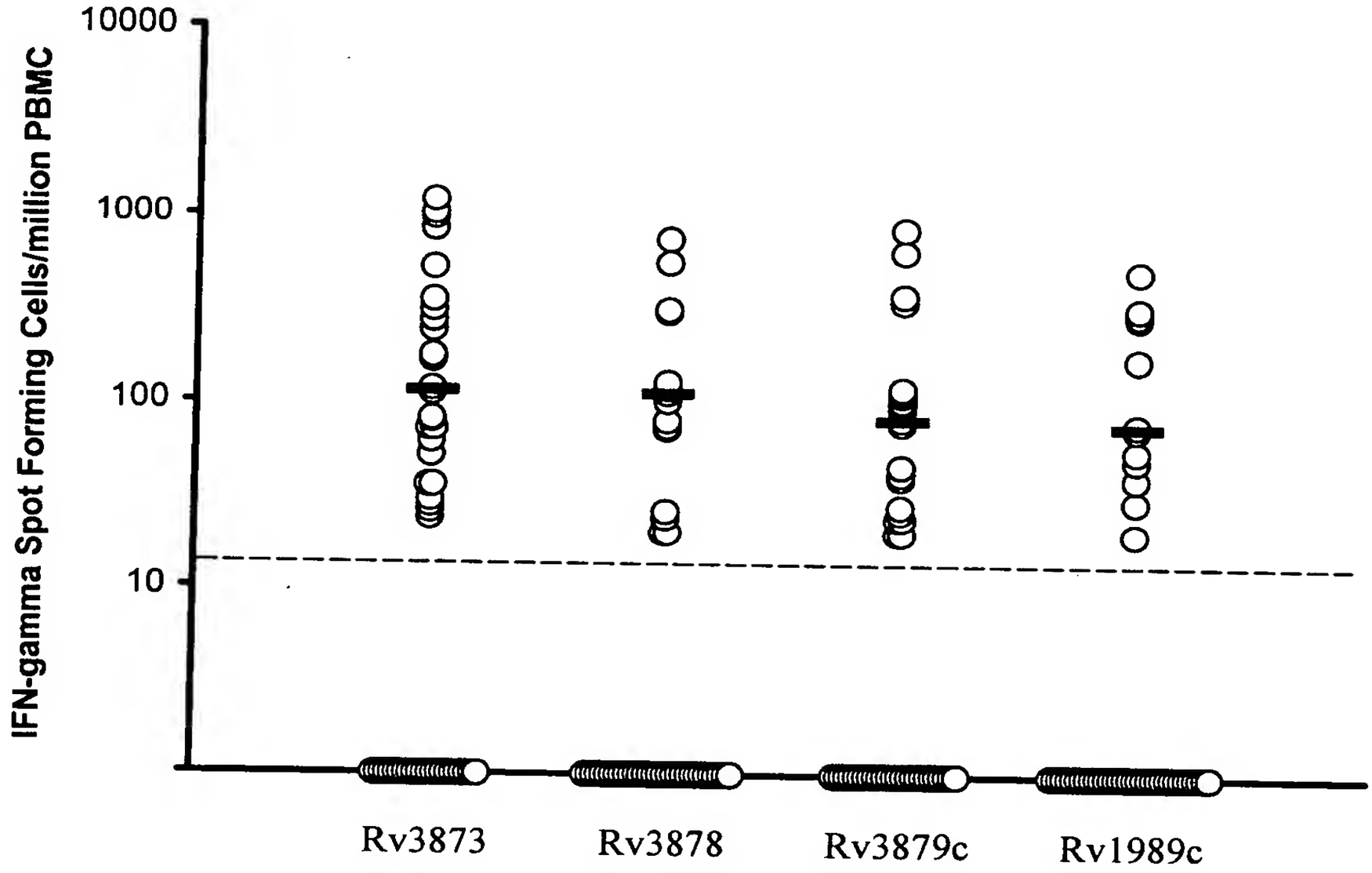

% subjects with positive response
90
80
70
60
50
40
30
20
10
0
Rv 3873 (RD1)
Rv 3878 (RD1)
Rv 3879c (RD1)
Rv 1989c (RD2)
Total (all 4 Ags)
PPD

Figure 2

A.


10000
1000
100
10
IFN-gamma Spot Forming Cells/million PBMC
Rv3873
Rv3878
Rv3879c
Rv1989c

B.

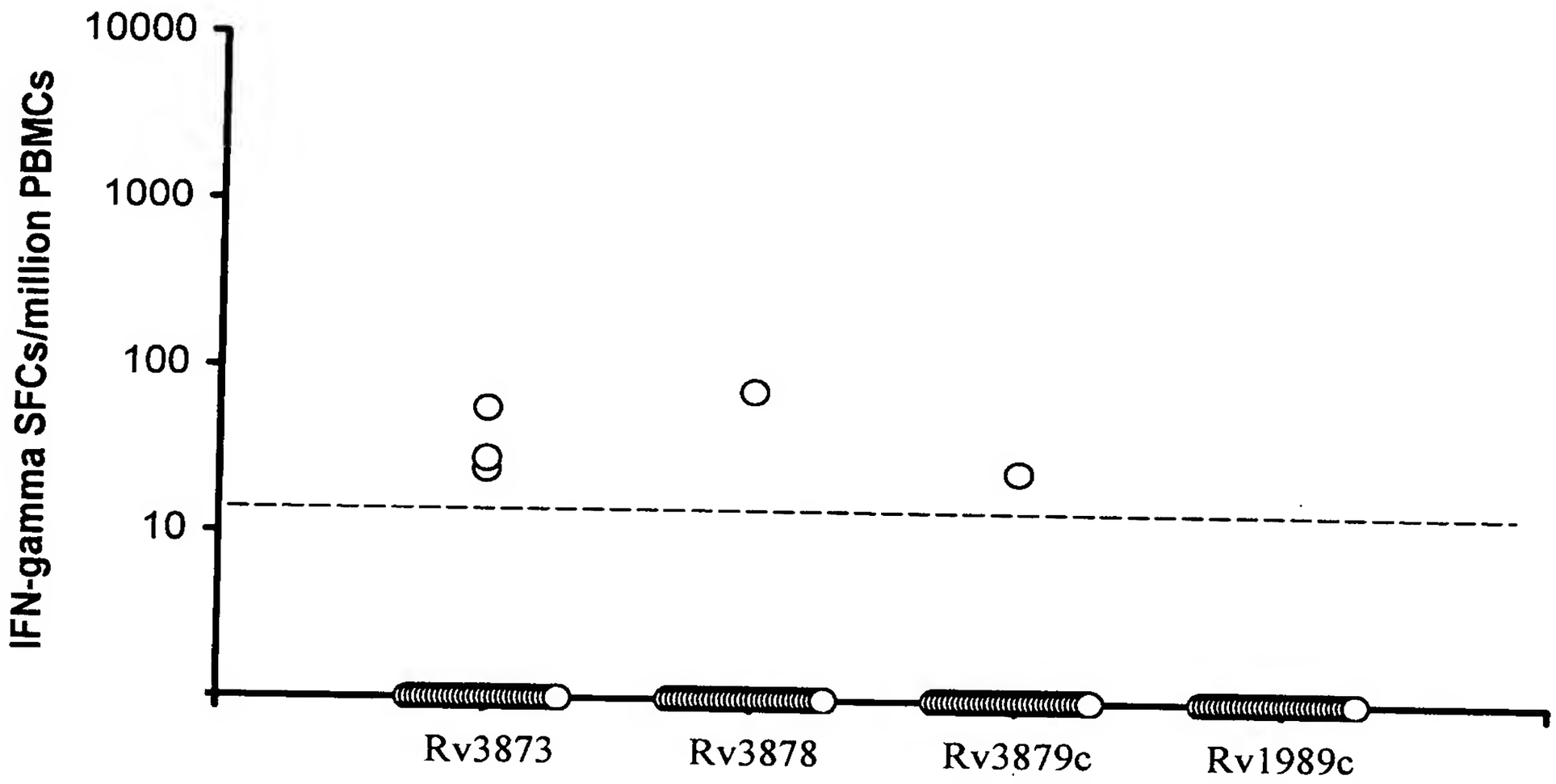

10000
1000
100
10
IFN-gamma SFCs/million PBMCs
Rv3873
Rv3878
Rv3879c
Rv1989c

Figure 3.

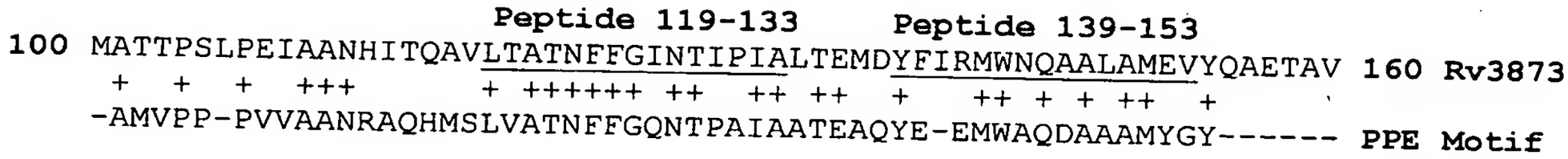


Figure 3

Location and homology of PPE protein family motif as described (http://genolist.pasteur.fr/TubercuLIST/mast/P210.1.html), within the partial amino acid sequence of Rv3873 (amino acid residues 100-160). Amino acid residues are shown in the one letter code. Underlined residues indicate the given peptide sequence. Identical residues are indicated with a cross.

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